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Water Stress and Damage Caused by *Puccinia jaceae* on Two *Centaurea* Species[1]

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The effect of water stress and disease damage to *Centaurea solstitialis* (yellow starthistle) and *C. calcitrapa* (purple starthistle) caused by isolates of the rust *Puccinia jaceae* was investigated under greenhouse conditions. Inoculated and noninoculated plants were placed under water stress, defined as reversible wilting; control plants received normal daily watering. Effects of drought stress and infection by *P. jaceae* were measured in terms of root biomass, lifespan of leaves with different disease severity levels, and the number of living and dead rosette leaves. Drought stress reduced root dry weights, leaf lifespan, and total number of rosette leaves of both yellow and purple starthistles. Infection by rust also reduced leaf lifespan and total rosette leaves of both species, but it only affected root biomass of yellow starthistle. The only significant interaction between drought stress and rust infection occurred with the leaf lifespan of purple starthistle, but this consisted of drought-stressed leaves having short lifespans at all infection levels. Results with *P. chondrillina*, a rust fungus that has been used successfully for biological control of *Chondrilla juncea* (rush skeletonweed) were similar to those of *P. jaceae* on yellow starthistle for root biomass and leaf lifespan. Data from these experiments with drought-stressed plants reinforced the previous conclusions that *P. jaceae* has promise as a biocontrol agent of yellow starthistle. The damage caused by *P. jaceae* was similar to that caused by *P. chondrillina* on skeletonweed in comparative studies.

KEY WORDS: biological control; *Centaurea solstilialis; Centaurea calcitrapa;* yellow starthistle; purple starthistle; drought stress; water stress; weed control; *Puccinia jaceae; Chondrilla juncea;* rush skeletonweed; *Puccinia chondrillina.*

INTRODUCTION

Classical biological control has considerable potential against introduced weeds of range and pastureland (Bruckart and Shishkoff, 1993; Watson, 1991). Both *Centaurea solstitialis* L. (yellow starthistle) and *C. calcitrapa* L. (purple starthistle) are weeds introduced from Mediterranean Eurasia that displace valuable range plants in the western United States (Amme, 1985; Maddox, 1981). Evaluations of the rust fungus, *Puccinia jaceae* Otth, for possible biological control of *Centaurea* spp. have been conducted under optimal conditions for disease development, including the inoculation of young, rapidly growing plants (Bruckart, 1989; Bennett *et al.*, 1991; Shishkoff and Bruckart, 1993); no stress factors beside disease have been deliberately applied. However, the effect of rust infection may be influenced by the water status of the host plant and vice versa. Rust infection is known to increase the transpiration rate of many plants by rupture of the epidermis during sporulation (Duniway and Durbin, 1971; Shtienberg, 1992). Bushnell and Rowell (1968) considered foliar rust infection to predispose wheat to drought stress by limiting root growth and function. Cowan and Van Der Wal (1975) found increased transpiration rates in rust-infected wheat; however, leaf-water potential was significantly lowered only in infected wheat grown at high soil-water potentials. They concluded that losses due to rust were minimized at "moderate" soil-water potentials (-400 J.Kg^{-1}). Paul and Ayres (1984) studied the effect of drought on groundsel (*Senecio vulgaris* L.) infected with *Puccinia lagenophorae* Cooke under laboratory conditions. Under well-watered conditions, rust-infected leaves showed reduced photosynthesis and increased dark respiration but young healthy leaves on the same plant

[1] Product names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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showed an increased rate of photosynthesis and an inhibition of photorespiration. Therefore, healthy leaves were able to compensate somewhat for losses due to infected leaves. Under drought conditions, rust-induced damage was increased largely due to increased transpiration. Both drought stress and rust infection reduced leaf size, but net photosynthesis was not reduced if rates were expressed per unit area of the entire plant. Although Paul and Ayres did not see an interaction between the reduction in leaf size caused by drought stress and rust infection, they suggested that the additive effect was sufficient to reduce the reproductive output of the plant.

Because starthistles are plants of dry rangeland, any interaction of drought stress and rust infection could be a significant factor in the success of a biological control program. Therefore, it might be advantageous to screen biological control candidates on plants grown under water-limiting conditions. The reproductive output of starthistles cannot be easily studied in the laboratory, since the flowers require outcrossing to produce fertile seed. However, the vigor of the plant prior to seed formation can be measured by weighing the root system, the site of carbohydrate storage in thistles. Previous research (Shishkoff and Bruckart, 1993) has shown that rust infection causes a significant reduction in root biomass in yellow starthistle, a winter annual of determinant growth habit, but not in that of purple starthistle, a bi- or triennial which remains in rosette form until it has sufficient reserves to bolt. The purpose of this research was to determine whether drought stress affected the damaging effects of rust infection on starthistles and whether the starthistle species differed in their response to drought stress. Also, the value of conducting evaluations of plant pathogens under normal greenhouse watering regimes was determined. An isolate of *Puccinia chondrillina* Bubak and Syd. that has been used successfully in biological control of *Chondrilla juncea* L. (rush skeletonweed) under field conditions (Cullen, 1986; Supkoff *et al.*, 1988) was included in these experiments for comparison.

MATERIALS AND METHODS

Sources and maintenance of rust fungi. All research was conducted in a containment greenhouse under permit from the USDA Animal and Plant Health Inspection Service (APHIS). The two *P. jaceae* isolates used in this study were collected in Turkey by S. Rosenthal in 1984 and were selected for detailed investigation from earlier studies (Shishkoff and Bruckart, 1993). Isolate YST71 was collected from yellow starthistle, and isolate PST66 was collected from purple starthistle. YST71 consistently reduced yellow starthistle root biomass, while PST66 did not reduce purple starthistle root biomass (Bennett *et al.*, 1991; Shishkoff and Bruckart, 1993). The isolate of *P. chondrillina* was collected from skeletonweed in Italy by L. Andres in 1975. Isolates were stored in liquid nitrogen for long term and in a refrigerator (4°C) for periods of 4 months or less. Frozen isolates were heated at 42°C for 6 min upon removal from liquid nitrogen (heat shocked) to stimulate germination.

Host propagation. Seeds of purple starthistle, yellow starthistle, and skeletonweed were collected in Solano County, Yolo County, and Placer County, California, respectively. Seeds were sown in 10-cm diameter clay pots filled with a pasteurized greenhouse mix of ProMix Bx (Premier Brands, Stamford, CT), soil, peat moss, vermiculite, perlite, and sand (4:2:2:2:1.5:1 v/v) with fertilizer (10:10:10,N:P:K) and wetting agent (AquaGro 2000-G, Aquatrol, Cherry Hill, NJ) added. Seedlings were thinned to 1 plant per pot for each experiment.

Inoculation of plants. When the entire aboveground portion of plants were inoculated, a simulated dry spore shower was generated using compressed CO_2 and the equivalent of 0.5 mg urediniospores per plant in a turntable settling tower while plants were rotating at 20 rpm (Melching, 1967). This resulted in deposition of 61 $\pm$ 13 spores/cm^2 of leaf surface, as determined by microscopic observation (Shishkoff, unpublished). Controls were not inoculated.

In experiments involving individual leaf inoculations, leaves were inoculated with 25 mg of urediniospores in 80 ml of an aqueous 0.13% polyoxyethylene sorbitan monolaurate (Tween-20) solution. Inoculum was applied to leaves by dipping a small polyurethane foam sponge into the spore suspension and then gently rubbing the leaf. Leaves of control plants were treated with the Tween 20 solution without spores.

All inoculated plants were placed in a darkened dew chamber (Lange *et al.*, 1958) for 12 to 15 h at 20°C. Untreated control plants were not placed in dew chambers, with the exception of control plants from single-leaf inoculation experiments, in order to avoid accidental contamination. After the dew period, all plants were placed on a greenhouse bench at 20 to 25°C for up to 5 weeks and observed for infection. Experiments were conducted from December 1991 to June 1992. Supplemental lighting with 400-watt sodium lamps was used in winter to provide a 16-h photoperiod.

Drought stress testing. The interaction of drought stress and rust infection on plants was measured in three ways; root biomass, leaf lifespan, and total leaf number.

In the root biomass experiments, 14 to 16 yellow starthistle, purple starthistle, or skeletonweed plants were inoculated twice, at 3 and 4 weeks after planting, using the turntable inoculation method with an equal number of plants serving as uninoculated controls.

After exposure to dew, these plants were arranged randomly on a greenhouse bench and incubated until early infection symptoms became visible on leaves, 2 to 4 days prior to pustule eruption. At this time, two watering regimes were implemented. Half of each treatment was watered according to the usual greenhouse routine of watering to saturation at least once a day. The other set of inoculated and uninoculated plants was subjected to reversible wilting by providing about 25% of the normal amount (60 ml/day). This resulted in daily drought stress lasting between 4 and 6 h in the afternoon. Plants were grown for 4 to 5 weeks under these conditions and then removed from the pots. Roots were washed to remove the soil, cut at the soil line from the shoots, dried at 30°C for 5 days, and weighed. This experiment was repeated once for each host/isolate combination.

To test the effect on leaf lifespan, 5-week-old plants of yellow starthistle, purple starthistle, and skeletonweed of similar size were selected. The single leaf on each plant selected for inoculation was directly adjacent to both a younger leaf not fully expanded and an older leaf of full size. The spore suspension described above was further diluted by 1/10 and 1/100, creating high-, medium-, and low-inoculum densities. For each experiment, two sets of 15 to 18 plants were inoculated on a single leaf. Roughly one-third of the plants were inoculated with each spore concentration and up to three plants were treated with the control solution. After 12 to 14 h of dew, plants were placed in the greenhouse and treated leaves were observed daily until flecks were visible, normally in 6 to 7 days. Then, as described, one set of plants was placed under drought stress and the other set was watered normally. After watering and reestablishment of turgor, plants were examined for dead, treated leaves. Leaves that had irreversibly lost turgor were considered "dead," thus "leaf lifespan" was arbitrarily defined as the number of days from inoculation until leaf death occurred. The number of pustules on dead, treated leaves was counted and the number of days from inoculation calculated. Lifespan of infected leaves was plotted against pustule number. Data were normalized using a log–log transformation and analyzed, as described below. The effect of drought stress on infected leaves was examined using analysis of covariance. This experiment was repeated four times with yellow starthistle and two times each with purple starthistle and skeletonweed.

To study the number of leaves produced by plants over time, 19 to 20 purple starthistle or yellow starthistle seedlings were grown under normal watering conditions for 2 weeks and then subjected to 9 weeks of drought stress, as described. An equal number of plants were watered normally. At 3 and 4 weeks after planting, half of the drought-stressed and normally watered plants were inoculated by the turntable method, described above, with the appropriate isolate. The other half served as uninoculated controls. Beginning at 2 weeks after planting, the number of living and dead leaves in each rosette was counted weekly.

Statistics. Data were analyzed using SAS software (SAS Institute, 1982). Root biomass data were tested by analysis of variance using PROC GLM. Data from the single-leaf inoculations were tested by analysis of covariance using GLM and SOLUTION procedures. Experiments on leaf lifespan were analyzed using both analysis of variance with repeated measurements in time (using the REPEATED and POLYNOMIAL procedures in SAS) and a split plot in time. UNIVARIATE and CORR procedures were used to determine normality and randomness (heterogeneity of residuals) of all datasets. Data not randomly distributed were log-transformed with an appropriate constant. Either Duncan's multiple-range test or least-square means test (LSMEANS) was used for separation of means. Treatments were considered to be significantly different at $P \geqslant 0.05$.

RESULTS

The results of experiments testing the effect of drought stress and rust infection on root biomass are summarized in Table 1. While skeletonweed and yellow starthistle were affected by both drought stress and infection, there was no interaction. Figure 1 illustrates the results of one yellow starthistle trial. Purple starthistle root biomass was affected only by drought stress.

In experiments in which single yellow starthistle leaves were inoculated, leaf lifespan significantly decreased with increasing pustule number. In three of five experiments, leaves of normally watered plants lived significantly longer at all levels of infection compared to leaves from drought-stressed plants. However, the interaction of drought stress and infection was not significant; in plots of leaf lifespan vs pustule number, slopes for drought-stressed and normally watered plants did not differ statistically according to analysis of covariance, indicating that there was no interaction (Fig. 2). In the remaining two experiments, drought stress did not significantly affect leaf lifespan.

For all three leaf lifespan experiments using skeletonweed, both drought stress and infection were significant factors, but there was no significant interaction.

Normally watered purple starthistle leaves lived significantly longer than leaves from drought-stressed plants in all three tests (Fig. 3). Pustule number per leaf was inversely proportional to leaf lifespan in watered plants, but drought-stressed leaves were very short-lived at all infection levels (Fig. 3). The results

TABLE 1

The Effect of Drought Stress and Rust Infection on Root Biomass of Three Weed Species, Yellow Starthistle (*Centaurea solstitialis*), Rush Skeletonweed (*Chondrilla juncea*), and Purple Starthistle (*Centaurea calcitrapa*) Subjected to Either or Both Stresses

	Weed species (no. of trials)		
Treatment	Yellow starthistle (2)	Skeletonweed (2)	Purple starthistle (2)
Drought stress	Root biomass significantly[a] reduced in both trials	Root biomass significantly reduced in both trials	Root biomass significantly reduced in both trials
Rust stress	Root biomass significantly reduced in one trial (P = 0.0004) and almost in the other (P = 0.06)	Root biomass significantly reduced in both trials	No significant effect on root biomass in either trial
Interaction of drought × infection	None	None	None

[a] Differences statistically significant ($P \geqslant 0.05$).

of experiments testing the effect of drought stress and rust infection on leaf number and leaf survival over time are summarized in Table 2.

Drought-stressed yellow starthistle plants produced fewer total (living and dead) leaves. Infection with the rust had no effect on total leaf number, and no significant interaction was seen for drought stress and inoculation. Drought stress did not influence the number of dead yellow starthistle leaves, but infection with the rust resulted in significantly more dead leaves regardless of water status (Fig. 4).

Fewer leaves were produced on drought-stressed purple starthistles, but infection had no effect on total leaf number. The number of dead leaves was greater on watered plants than on water-stressed plants, but there was no significant increase in the number of dead leaves on plants infected with *P. jaceae* (Fig. 5).

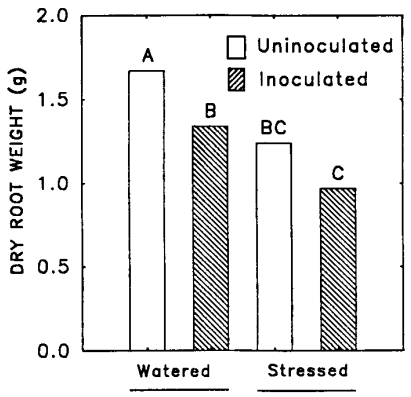

FIG. 1. Dry root weight (g) of yellow starthistle plants inoculated (hatched bars) or not inoculated (open bars) with *Puccinia jaceae* and then drought-stressed or watered normally. Bars with same letters are not significantly different (P = 0.05) according to analysis of variance.

DISCUSSION

Results from drought-stressed plants agreed with data from our previous studies with well-watered yellow and purple starthistles infected with *P. jaceae* (Shishkoff and Bruckart, 1993). This implies that, under greenhouse conditions, the inclusion of drought stress as a variable did not alter our conclusions about the potential of a given rust isolate as a biocontrol agent. The yellow starthistle isolate of *P. jaceae* consistently reduced yellow starthistle root biomass, while the purple starthistle isolate did not affect purple star-

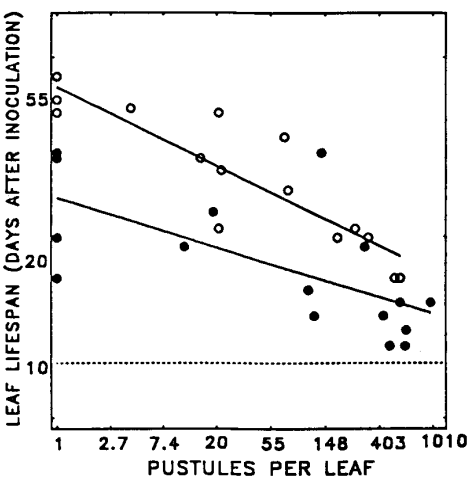

FIG. 2. Log–log plot of leaf lifespan (days) vs pustule number on yellow starthistle inoculated on single leaves with *Puccinia jaceae*, when plants were drought-stressed (filled circles) or watered normally (open circles). Slopes of the lines did not differ significantly (P = 0.05) by analysis of covariance. The dotted line indicates the time of pustule eruption. Each dot or circle represents a single plant. Data are from one representative experiment.

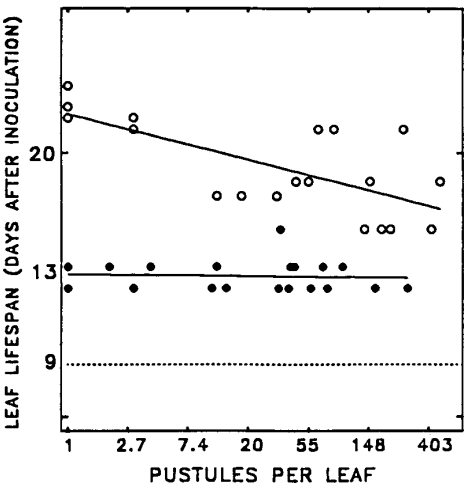

FIG. 3. Log–log plot of leaf lifespan (days) vs pustule number on purple starthistle inoculated on single leaves with *Puccinia jaceae*, when plants were drought-stressed (filled circles) or watered normally (open circles). Slopes of the lines were significantly different ($P = 0.05$) by analysis of covariance. The dotted line indicates the time of pustule eruption. Each dot or circle represents a single plant. Data are from one representative experiment.

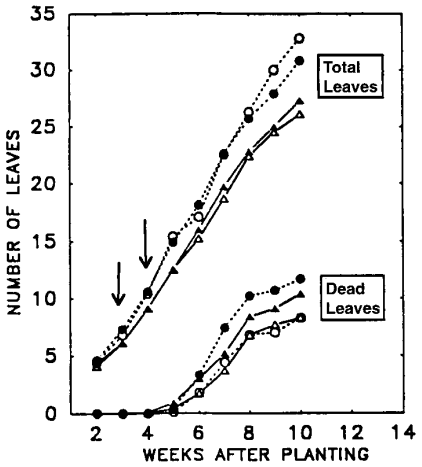

FIG. 4. Total number of leaves developing over time vs dead leaves accumulating over time on yellow starthistle rosettes inoculated (arrows) at 3 and 4 weeks (filled symbols) or not inoculated (open symbols), and drought-stressed (triangles) or watered normally (circles). Each treatment combination represents the mean of 10 plants. The number of dead leaves was significantly greater ($P = 0.05$) on inoculated plants than uninoculated controls, regardless of watering scheme.

thistle root biomass, despite similar levels of disease severity as measured by pustule counts (Shishkoff and Bruckart, 1993). This agrees with other studies showing that visible symptoms caused by foliar fungal pathogens do not necessarily correlate with damage or physiological responses to disease (Shtienberg, 1992).

With yellow starthistle, there was no interaction between drought stress and infection under greenhouse conditions. Under normal watering conditions or under drought stress, pustule number was inversely proportional to leaf lifespan. With purple starthistle, an interaction was observed, but it consisted of drought-stressed leaves dying rapidly regardless of infection amount. From these data, we cannot conclude that the purple starthistle rust isolate will be more effective under dry field conditions. In all evaluations, however, the yellow starthistle isolate consistently showed promise as a biological control agent.

That the results differed according to the host system tested was not unexpected; previous research on these pathosystems suggested that differences might be related to characteristics of the plant host at similar levels of disease severity rather than to differences in virulence of rust isolates (Shishkoff and Bruckart, 1993). It was apparent that uninfected yellow starthistle leaves lived significantly longer than purple starthistle

TABLE 2

The Effect on Yellow Starthistle (*Centaurea solstitialis*) and Purple Starthistle (*Centaurea calcitrapa*) of Drought Stress (Initiated at 2 Weeks after Planting) and Rust Infection (Plants Inoculated at 3 and 4 Weeks after Planting) Measured as the Increase in Total (Living and Dead) Rosette Leaves and Dead Rosette Leaves over a 9-week Period

	Yellow starthistle		Purple starthistle	
Treatment	Total no. of leaves	No. of dead leaves	Total no. of leaves	No. of dead leaves
Drought stress	Significant[a] reduction in total leaf no.	No effect on leaf death rate	Significant reduction in total leaf no.	Significant reduction in leaf death rate
Rust infection	No effect on total leaf number	Significantly increased death rate	No effect on total leaf number	No effect on leaf death rate
Interaction of drought × infection	None	None	None	None

[a] Differences statistically significant ($P \geqslant 0.05$).

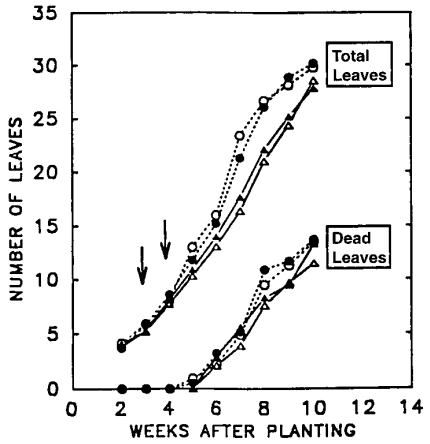

FIG. 5. Total number of leaves developing over time vs dead leaves accumulating over time when purple starthistle rosettes inoculated (arrows) at 3 and 4 weeks (filled symbols) or not inoculated (open symbols), and drought-stressed (triangles) or watered normally (circles). Each treatment combination represents the mean of 10 plants. Differences between treatments were not statistically significant ($P = 0.05$).

leaves, and reduction in leaf lifespan in infected yellow starthistle was proportionally greater than that observed for infected purple starthistle (Shishkoff and Bruckart, 1993). Yellow starthistle has a determinate number of leaves in its rosette, and one would, therefore, expect each leaf to be relatively more valuable to the plant in contributing photosynthate than each leaf of purple starthistle, which is rapidly cycled and short-lived.

Differences in host plant response makes predictions from one host–pathogen system to another problematic. Bushnell and Rowell (1968), working with wheat plants infected with *P. graminis* Pers.: Pers. f. sp. *tritici* Eriks. & E. Henn, considered the decline in root growth caused by rust infection to be due to decreasing translocation of food or vitamins from leaves. Subsequent death of shoots was attributed to water stress caused by decreased root size and function. Similarly, we speculate that the determinate rosette of yellow starthistle limits its ability to outgrow rust infections since it must complete its life cycle in 1 year; the reduction of root biomass by rust infection reduces the amount of carbohydrates available for bolting and seed production. Shoot death was never observed with *Centaurea,* but Bushnell and Rowell (1968) observed shoot death at "extremely heavy rust infections." Such heavy infections were not induced in these experiments with starthistles and skeletonweed, nor would high infection levels be expected in the field. Duniway and Durbin (1971) considered rust infection to exacerbate drought stress in bean plants through increased transpiration. Our data showed a decrease in leaf lifespan with increasing pustule number, but lifespan did not decrease more rapidly in drought-stressed plants. This is contrary to what one would expect if increased transpiration from rust infection were exacerbating drought stress.

Obviously, rust infection and drought stress are only two factors out of many affecting plant growth. The effect of rust on the competitive ability of grounsel depended on the water status; when water was limiting, the reduction in competitive ability was more pronounced (Paul and Ayres, 1984). Paul *et al.* (1993) suggested that under natural conditions rust-infected grounsel would be influenced by many factors, including soil-water potential, host nutrient status, infection of the plant by secondary invaders, and inter- and intraspecific competition. This highlights the importance of field testing, since it is impossible to include all these factors in a screening program.

The timing of such factors might also be important. The ideal conditions for rust infection in California's Central Valley to plant growth occur in late spring, when adequate dew is present for infection and temperatures are warm enough to encourage secondary spread of the fungus. By summer, dew periods are insufficient to allow infection, and temperatures could be high enough to inhibit growth of the fungus. Therefore, if rust infection is to reduce the reproductive output of the plant, it must reduce the plant's growth dramatically by spring's end, or there must be long-lasting effects that carry over through summer, such as increased transpiration from infected leaves, secondary invaders, or interspecific competition that does not allow the plant to regain its vigor.

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REFERENCES

Amme, D. 1985. Controlling purple star thistle: A case study. *Fremontia* 13, 22–23.

Bennett, A. R., Bruckart, W. L., and Shishkoff, N. 1991. Effects of dew, plant age, and leaf position on the susceptibility of yellow starthistle to *Puccinia jaceae. Plant Dis.* 75, 499–501.

Bruckart, W. L. 1989. Host range determination of *Puccinia jaceae* from yellow starthistle. *Plant Dis.* 73, 155–160.

Bruckart, W. L., and Shishkoff, N. 1993. Foreign plant pathogens for environmentally safe biological control of weeds. *In* "Pest Management with Biologically Based Technology. Beltsville Agricultural Symposium—18" (R. D. Lumsden and J. L. Vaughan, Eds.). Am. Chem. Soc., Washington, DC.

Bushnell, W. R., and Rowell, J. B. 1968. Premature death of adult

rusted wheat plants in relation to carbon dioxide evolution by root systems. *Phytopathology* 58, 651–658.

Cowan, M. C., and Van Der Wal, A. F. 1975. An ecophysiological approach to crop losses, exemplified in the system wheat, leaf rust and glume blotch. IV. Water flow and leaf-water potential of uninfected wheat plants and plants infected with *Puccinia recondita* f.sp. *triticina. Neth. J. Pl. Pathol.* 81, 49–57.

Cullen, J. M. 1986. Bringing the cost benefit analysis of biological control of *Chondrilla juncea* up to date. "Proc. VI Int. Symp. Biol. Contr. Weeds" *In* (E. S. Delfosse, Ed.), pp. 145–152. Agr. Canada, Ottawa.

Duniway, J. M., and Durbin, R. D. 1971. Some effects of *Uromyces phaseoli* on the transpiration rate and stomatal response of bean leaves. *Phytopathology* 61, 114–119.

Lange, C. T., Kingsolver, C. H., Mitchell, J. E., and Cherry, E. 1958. Determination of the effects of different temperatures on uredial infection with *Puccinia graminis* var. *tritici. Phytopathology* 48, 658–660.

Maddox, D. M. 1981. "Introduction, Phenology, and Density of Yellow Starthistle in Coastal, Intercoastal, and Central Valley Situations in California." USDA, ARS, Agricultural Research Results, Western Series, No. 20, July 1981.

Melching, J. S., 1967. Improved deposition of airborne uredospores of *Puccinia graminis* and *P. striiformis* on glass slides and on wheat leaves by use of a turntable. (Abstr.) *Phytopathology* 57, 647.

Paul, N. D., and Ayres, P. G. 1984. Effects of rust and post-infection drought on photosynthesis, growth and water relations in groundsel. *Plant Pathol.* 33, 561–569.

Paul, N. D., Ayres, P. G., and Hallett, S. G. 1993. Mycoherbicides and other biocontrol agents for *Senecio* spp. *Pestic. Sci.* 37, 323–329.

SAS Institute, 1982. SAS User's Guide: Statistics. SAS Institute, Cary, NC.

Shishkoff, N., and Bruckart, W. 1993. Evaluation of infection of target and nontarget hosts by isolates of the potential biocontrol agent, *Puccinia jaceae* that infect *Centaurea* spp. *Phytopathology* 83, 894–898.

Shtienberg, D. 1992. Effects of foliar diseases on gas exchange processes: A comparative study. *Phytopathology* 82, 760–765.

Supkoff, D. M., Joley, D. B., and Marois, J. J. 1988. Effect of introduced biological control organisms on the density of *Chondrilla juncea* in California. *J. Appl. Ecol.* 25, 1089–1095.

Watson, A. K. 1991. The classical approach with plant pathogens. *In* "Microbial Control of Weeds" (D. O. TeBeest, Ed.), pp. 3–23. Chapman and Hall, New York.